

The University of Chicago

**SUPPOSED ROLE OF THE ADRENALS
IN HYPERTENSION**

**I. QUANTITATIVE STUDIES ON EFFECTIVE EPINEPHRIN
CONCENTRATIONS IN SYSTEMIC BLOOD AND THEIR
RELATION TO EPINEPHRIN OUTPUT FROM
THE ADRENALS**

**II. QUANTITATIVE STUDIES ON LOSS OF EPINEPHRIN
FROM CIRCULATING BLOOD**

**A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF PHYSIOLOGY

1937

By

EMANUEL MARCUS

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IN SYSTEMIC BLOOD AND THEIR RELATION TO EPINEPHRIN
OUTPUT FROM THE ADRENALS

INTRODUCTION

From clinical and experimental evidence that has accumulated since Thomas Addison first called attention to the syndrome which results from disease of the adrenals, a direct relationship between the function of these glands and blood pressure phenomena has been believed to exist. Primarily, such a relationship is based on two important observations: (a) that blood pressure falls when the adrenals are diseased beyond functional integrity (e.g., Addison's disease) or when an animal is deprived of both adrenals surgically, and (b) that a powerful pressor influence is exerted by intravenous injection of aqueous or saline extracts of the adrenal medulla or solutions of its product, epinephrin, in pharmacological quantities.

It is assumed, chiefly from this evidence, that normal blood pressure is dependent upon an influence of epinephrin secreted from the adrenals and that hypertension, occurring in various diseases, is related to increased epinephrin secretion. While this concept still prevails among many clinicians and some physiologists, there is substantial evidence that the epinephrin ordinarily secreted from the adrenals does not play a significant role in the maintenance of normal blood pressure.

Elliott (1), Stewart and Rogoff (2) and others have demonstrated that reduction or suppression of epinephrin secretion from the adrenals, by excision of one adrenal and denervation of the other, in experimental animals, is not incompatible with life and good health. No significant change in blood pressure occurs in such animals. Furthermore, Rogoff and Dominguez (3) found that complete bilateral adrenalectomy does not lead to a fall in blood pressure until some days after the adrenals have been excised, i.e., not until manifestations of adrenal cortical insufficiency develop. Young and Lehman (4) and later Hoskins and

McClure (5) observed no fall in blood pressure on ligation of the adrenals.

It seems well established that epinephrin from the adrenals is not essential for maintenance of normal blood pressure. There is not enough experimental evidence of a quantitative nature, however, to support the view that epinephrin secreted by the adrenals plays an important part in causing or maintaining elevated blood pressure under various abnormal conditions, viz., functional changes in blood pressure during emotional excitation, or hypertension associated with various diseases. Cannon and de la Paz (9) have suggested the following:

Injected epinephrin is capable of inducing an atheromatous condition of the arterial wall in rabbits, especially in elderly individuals, and is also capable of evoking hyperglycemia and glycosuria. As Ascher has shown, by prolonged stimulation of the splanchnic nerves, prolonged secretion of epinephrin with maintained high blood pressure can be produced. In the light of the results here reported [increased epinephrin secretion due to emotional excitation] the temptation is strong to suggest that some phases of these pathologic states are associated with the strenuous and exciting character of modern life acting through the adrenal glands. This suggestion, however, must be put to experimental test.

The idea thus elaborated in 1911 still prevails and has constituted a basis for treatment of hypertension in human beings by roentgen irradiation of the adrenals, partial adrenalectomy, and/or denervation of the glands. Since these therapeutic procedures involve the possible danger of serious damage to the indispensable adrenal cortex, which may result in chronic adrenal insufficiency (Rogoff, 6) or in death, it seemed important to make suitable quantitative studies to determine, if possible, to what extent, if any, epinephrin may participate in the etiology of hypertension.

From available evidence, one must assume that in hypertension and related conditions epinephrin is present in the systemic circulation in such concentrations that it should be possible to detect it with available test objects. Stewart (7) was unable to detect epinephrin in systemic blood under these conditions. Rogoff and Wasserman (8) were unable to find detectable amounts of epinephrin in systemic blood obtained from patients with essential hypertension. They also made studies by injecting various amounts of epinephrin in single doses and by constant injection, to elicit information concerning the relation of such quantities to blood pressure reactions. It was aimed to determine

what concentrations of epinephrin in systemic blood are necessary to produce definite effects. Their experiments were performed on cats, anesthetized with urethane. From their observations, as well as from experiments on dogs here reported it seems evident that such quantities of epinephrin as would be required to maintain blood pressure at a significantly elevated level would bring the epinephrin concentration in the systemic blood up to a level that should easily be detected by sensitive segments of rabbits' intestine. Accordingly, the study of this question was undertaken as a part of the series of studies outlined by Dr. J. M. Rogoff on this subject.

It has been suggested by Cannon and de la Paz (9) that epinephrin, set free by nervous stimulation during emotional excitation, may return to the glands in the blood stream and stimulate them to further secretion. If this occurs, it is conceivable that it could constitute an underlying factor in the production of a persistent elevation of blood pressure.

EXPERIMENTAL

Experiments were undertaken to test this question by actual measurement of the epinephrin output from the adrenals. Intravenous injections of epinephrin were made with various doses. In three experiments the epinephrin was administered in a single injection, to imitate a sudden "outburst" that might occur if the concept of an emergency function is valid. In other cases epinephrin was administered by constant injection at a uniform rate, during 1 to 20 minutes. Adrenal vein blood specimens were obtained via the "cava pocket" and assayed for epinephrin concentration on rabbits' intestine segments, according to the method of Stewart and Rogoff.

Technique

All constant injections were made by means of a constant injection machine (designed by J. M. Rogoff) calibrated to deliver anywhere from 0.7 to 7.5 cc. per minute at a perfectly constant flow against any pressure met intravascularly. The essential construction of the apparatus includes a motor that drives a muddled disc by a friction (hard rubber) drive; the disc operates a pinion and gear reduction to a screw which is kept in con-

stand motion, propelling forward one, two, or three syringes.

All the bloods, immediately upon withdrawal, were defibrinated in porcelain crucibles with glass stirring rods, transferred to centrifuge tubes and placed in a refrigerator until just before the assay, at which time they were surrounded by ice for use during the assay. The cava pocket specimens were weighed immediately upon withdrawal. The peripheral blood specimens were always centrifuged, and serum used for assay, making the detection of lower concentrations of epinephrin easier, since all epinephrin in blood is present in the serum (Stewart and Rogoff, 10).

All specimens were assayed by the rabbit intestine segment method of Stewart and Rogoff. In three experiments (90, 102, 103) the cat sensitized pupil method, as recently described by J. M. Rogoff (11) was also employed to confirm the assay on the intestine segment, and the two methods yielded results in excellent agreement in all cases.

A. EXPERIMENTS ON THE RATE OF LIBERATION OF EPINEPHRIN FROM THE ADRENALS

Condensed protocols of experiments on effect of single injection of a large dose of epinephrin

Dog #56, M, 10.25 kg. Nembutal anesthesia. Adrenal vein blood specimen I obtained; rate of blood flow, 9.65 gms/min. B.P. 104 mm. Hg. Intravenous injection of 1.0 mgm. epinephrin HCl (this would correspond to 500 times the average normal output if it were injected constantly over 1 min.). Maximum B.P. rise to 225 mm. Hg. Adrenal blood specimen II obtained during ascent of B.P.; rate of blood flow--10.0 gms/min. Specimen I was not assayed satisfactorily. Specimen II was stronger than 1:1,000,000, and weaker than 1:500,000 epinephrin; it was assayed at 1:750,000, which would correspond to an output of 0.014 mgms. of epinephrin per minute for the dog, or 0.0014 mgms/kg/min. Although specimen I was not quantitated, it was decidedly weaker than II.

Dog #73, F, 8.5 kg. Urethane anesthesia. B.P.--80 mm. Hg. Adrenal vein blood specimen I obtained; rate of blood flow, 16.9 gms/min. Injection of 0.5 mgm. of epinephrin HCl (this would correspond to 300 times the average normal output if it had been injected constantly over 1 min.). Maximum rise in B.P. to 188 mm.

Hg. Adrenal vein blood specimen II obtained as soon as B.P. returned to 56 mm. Hg.; rate of blood flow, 12.25 gms/min. Adrenal vein blood specimen III obtained 15 min. after collection of specimen II; rate of blood flow--13.2 gms/min. and B.P.--58 mm. Hg. Specimen I was assayed at 1:4,000,000 epinephrin, yielding an output of 0.0039 mgm. per minute for the animal or 0.00047 mgms/kg/min. Specimen III was assayed at 1:4,000,000--yielding an output of 0.0032 mgs/min. for the animal or 0.00038 mgms/kg/min. Specimen II was assayed at 1:4,000,000--corresponding to an output of 0.0030 mgs/min. for the dog or 0.00036 mgms/kg/min.

Dog #74, M, 9.5 kg. Urethane anesthesia. Adrenal vein blood specimen I (B.P.--88), obtained; blood flow--11.4 gms/min. B.P.--75 mm. Hg. Injected 0.8 mgm. epinephrin HCl (this would correspond to 450 times the average normal output if it had been injected constantly over 1 min.). Specimen II taken at the height of B.P. rise which was to 175 mm. Hg.; blood flow--16.6 gms/min. Adrenal vein blood specimen III obtained 7 min. after fall of pressure to 67 mm. Hg.; blood flow--7.1 gms/min. Specimen I was assayed at 1:5,000,000--yielding an output of 0.00228 mgms/min. for the dog, or 0.00024 mg/kg/min. Specimen II was assayed at 1:3,000,000--corresponding to an output of 0.0055 mgs/min. for the dog, or 0.00057 mgm/kg/min. Specimen III was assayed at 1:4,000,000--yielding an output of 0.00187 mgms/min. for the dog, or 0.00018 mgm/kg/min.

Condensed protocols of experiments on effect of constant injection of a large dose of epinephrin

Dog #104, F, 8.9 kg. Urethane anesthesia. Injected 1.08 mgms. epinephrin HCl constantly over 2 min. (corresponds to 300 times the normal rate of output). B.P. 95 mm. Hg. adrenal vein blood specimen I was obtained at the peak of B.P. rise--205 mm. Hg. blood flow--14.0 gms/min. Specimen II was an arterial specimen taken from the aorta simultaneously with collection of adrenal vein specimen I. B.P. fell to 80 to 88 mm. Hg. Specimen I was assayed at 1:1,500,000--corresponding to an output of 0.00938 mgms/min. for the dog, or 0.00105 mg/kg/min. Specimen II was assayed at 1:1,000,000.

Dog #75, M, 9.5 kg. B.P. 68 mm. Hg. adrenal vein specimen I obtained, blood flow--6.5 gm/min. Injection of 2.0 mgms. epinephrin HCl constantly in a period of 2 min. (this corresponds to

500 times the normal output from the adrenals). Adrenal vein blood specimen II obtained 30 seconds after end of the injection, while the B.P. was still at its peak of 185 mm. Hg., blood flow--15.5 gm/min. Adrenal vein blood specimen III taken 5½ min. after II, with B.P. at 40 mm. Hg. blood flow--2.33 gm/min. Specimen I was assayed at 1:3,000,000--yielding an output of 0.00209 mgm/min. for the dog, or 0.00022 mgm/kg/min. Specimen II assayed at 1:500,000, corresponding to an output of 0.0285 mgm/min. for the dog, or 0.003 mg/kg/min. Specimen III assayed at 1:5,000,000, yielding an output of 0.000475 mgm/min. for the dog, or 0.00005 mgm/kg/min.

Dog #110, F, 9.65 kg. Urethane anesthesia. Injection of 0.772 mgms. epinephrin HCl constantly over 2 min. (this corresponds to 200 times the normal rate of epinephrin output from the adrenals). Adrenal vein blood specimen I obtained immediately after end of injection; blood flow--12.3 gms/min. B.P. from 210 to 170 mm. Hg. Specimen II was collected from the abdominal aorta and was taken 30 seconds after collection of I, when B.P. was 120 mm. Hg. Specimen I was assayed at 1:4,000,000--yielding an output of 0.00288 mgm/min. for the dog, or 0.0003 mgm/kg/min. Specimen II was assayed at 1:10,000,000.

Dog #109, F, 9.35 kg. Urethane anesthesia. B.P. 100 to 104 mm. Hg. Injected 0.187 mgms/min. Adrenal vein blood specimen I, blood flow of 13.98 gms/min., taken 6 min. after beginning of the injection (while the injection was still going on). B.P. 170 (maximal) mm. Hg. Specimen II was taken from the aorta simultaneously with collection of adrenal specimen I. Specimen I was assayed at 1:1,000,000--yielding an output of 0.0139 mgm/min. for the dog, or 0.0014 mgm/kg/min. The epinephrin concentration in specimen II was found to be very slightly less than in specimen I.

Condensed protocols of experiments on effect of constant injection of a small dose

Dog #48, 10.25 kg. Nembutal anesthesia. B.P. not recorded. Adrenal vein blood specimen I obtained; blood flow, 14.6 gms/min. Injected 0.04 mgm. constantly over 1 min. (corresponds to 20 times normal rate of output from the adrenals). Adrenal vein blood specimen II taken immediately after end of the injection; blood flow, 10.5 gms/min. Twenty-five minutes later the same injection was repeated and specimen III, like specimen II, obtained just

after end of the injection; blood flow, 8.25 gms/min. Specimen I assayed at 1:6,500,000--yielding an output of 0.0022 mgm/min. for the dog, or 0.0002 mgm/kg/min. Specimen II assayed at 1:5,000,000, yielding an output of 0.0021 mg/min. for the dog, or 0.0002 mgm/kg/min. Specimen III was assayed at 1:4,000,000--yielding an output of 0.0021 mg/min. for the dog, or 0.0002 mgm/kg/min.

Dog #49, 8.75 kg. Nembutal anesthesia. Adrenal vein blood specimen I obtained; blood flow, 10.7; B.P. 132 mm. Hg. Injection of 0.036 mgm. constantly over 1 min. (corresponding to 20 times normal rate of output from the adrenals). Adrenal vein blood specimen II, blood flow, 9.5, collection begun in the middle of the injection and continued until 30 seconds after the end of the injection; B.P. 140 mm. Hg. to 156 mm. Hg. Specimen I assayed at 1:50,000,000--yielding an output of 0.00021 mgm/min. for the dog, or 0.000024 mgm/kg/min. Specimen II assayed at 1:20,000,000--corresponding to an output of 0.00045 mgm/min. for the dog, or 0.000052 mgm/kg/min.

Dog #51, F, 7.0 kg. Nembutal anesthesia. B.P. 112 mm. Hg. Injection of 0.07 mgm/min. (corresponding to 50 times the normal rate of output from the adrenals). Adrenal vein blood specimen I, 6.2 gms/min.--taken 10 min. after beginning of the injection (with the injection still in progress); B.P. 170 to 182 mm. Hg. Specimen II, 9.6 gms/min. taken 20 min. after beginning of the injection (with the injection still in progress); B.P. 142 mm. Hg. Specimen I assayed at 1:4,000,000--yielding an output of 0.00155 mgm/min. for the dog, or 0.00022 mgm/kg/min. Specimen II assayed at 1:5,000,000--corresponding to an output of 0.00192 mgm/min. for the dog, or 0.00027 mgm/kg/min.

Dog #77, M, 14.1 kg. Urethane anesthesia. Adrenal vein blood specimen I, blood flow, 22.0; B.P. 110 mm. Hg. Injected 0.028 mgm/min. for 3 min. (corresponding to 10 times the normal rate of output from the adrenals). Adrenal vein blood specimen II, blood flow, 13.9 gms/min. taken immediately after end of the injection; B.P. 108 to 94 mm. Hg. Maximum rise of B.P. was 118 mm. Hg. No quantitative assay, but qualitatively II was slightly greater than I as one would expect from the difference in blood flows, if the output remained the same.

Discussion of the Results of the Experiments on Liberation of Epinephrin

From these experiments it is evident that administration of epinephrin, in a single dose or injected constantly, does not significantly influence the rate of liberation of epinephrin from the adrenal glands. In the experiments with the smaller amounts of injected epinephrin (corresponding to ten to fifty times the ordinary average epinephrin output from the adrenals per minute) not only was there no evidence that the rate of liberation of epinephrin from the glands was altered, but even the concentration of epinephrin in the adrenal vein blood showed no evidence of presence of excess of epinephrin. This indicates that, although these quantities of epinephrin are capable of affecting the blood pressure, quite markedly with the higher doses, this effect is transient and no influence is being exercised on the rate of liberation of epinephrin from the adrenals during or following the pressor action yielded by the injected epinephrin.

It should be emphasized that the amounts of epinephrin above referred to as "smaller" are in reality very large when compared with the maximum amounts of epinephrin liberated from the adrenals under the influence of powerful stimulation (e.g., stimulation of splanchnic nerves, action of certain drugs, strychnine and nicotine). With the larger doses employed in these experiments the results obtained must be interpreted with caution. On superficial examination it might appear that the rate of liberation of epinephrin from the adrenals was augmented. Such an interpretation, however, is not warranted. Although the calculated rate of output would indicate an increased liberation, the apparent increase can readily be accounted for by the increased epinephrin concentration in the adrenal vein blood which merely reflects the increased concentration of epinephrin in the arterial (systemic) blood due to the tremendous amount of epinephrin injected. This is to be expected inasmuch as the adrenal vein blood is largely arterial because of the very high rate of blood flow through the adrenals. In these experiments, it is seen, as is shown later in the experiments on the loss of epinephrin in the blood after injection, even with massive doses such as have been employed, the

epinephrin disappears rapidly from the circulation. The injected epinephrin is lost so rapidly from the circulating blood that the experimental value for epinephrin concentration in systemic blood never approaches the value calculated on the basis of total amount injected, if the specimen is collected at the height of the blood pressure rise. However, if the specimen is collected on the ascent of the blood pressure (#56, #49), at a time when the injected epinephrin has not yet been utilized in eliciting the response, the experimental concentration in the systemic blood does approach the calculated value. This observation indicates that some epinephrin is lost from the circulating fluid on the very first circuit, and agrees with a similar one by Rogoff and Wasserman.

Another point of interest was observed in some experiments with the large doses. Adrenal blood collected within a few minutes after the end of epinephrin injection not only fails to show the aforementioned "apparent" increase, but there is evidence that these large doses of epinephrin may actually inhibit epinephrin liberation from the adrenals to a certain extent, for a time. Thus: In dog #75, specimen II, collected from the adrenals 30 seconds after end of injection and while the blood pressure was still at its peak, showed a concentration of 1:500,000--yielding an "apparent" output of 0.003 mgm/kg/min. Theoretically the amount of epinephrin injected should have yielded a concentration in arterial blood of approximately 1:300,000, without considering the additional epinephrin contributed by the adrenals. This indicates that within 30 seconds about one-half of the injected epinephrin was already lost, and corresponds with the results obtained by Rogoff and Wassermann on cats. Specimen III, obtained from the adrenals 5½ minutes after specimen II was collected, when the blood pressure had fallen to 40 mm. Hg., contained only one-tenth the concentration of epinephrin that was found in specimen II. This corresponds with about one-fourth the rate of output before the large dose of epinephrin was injected.

The evidence in the literature for increased epinephrin liberation following intravenous administration of epinephrin can be considered untenable. Some of the evidence is based on work with methods inadequate for satisfactory quantitative information. Elliott (1) assumed an increased liberation of epinephrin from the adrenals on the basis of a diminished epinephrin store. Such a conclusion cannot be considered unequivocal since the store

merely represents a balance between production and liberation of epinephrin. Indeed, the illustration given (dog #75) indicates the probability that an inhibitory influence, perhaps exerted on the mechanism for production of epinephrin, might account for the reduced output found in specimen III, as well as for the reduced epinephrin store found by Elliott.

From the evidence at hand, it seems justifiable to conclude that an increased amount of circulating epinephrin does not stimulate the adrenals to further secretion of epinephrin.

B. EXPERIMENTS ON THE LOSS OF EPINEPHRIN FROM THE CIRCULATING BLOOD

It is conceivable that if epinephrin is retained in the circulation or in the tissues it might accumulate in sufficient quantities to yield pressor or other effects when released. This possibility was investigated, in cats, by Rogoff and Wasserman (1932; 8), and further studies were included in the present investigation, in dogs.

In the work on cats single doses of epinephrin were administered in quantities corresponding to as much as 25 times the normal output per minute from the adrenals. However, since a single injection requires only about 10 seconds, this would really correspond to 150 times normal for a period of 10 seconds. With these single injections they found that a specimen of systemic blood, obtained within a period which corresponds to the time necessary for one complete circuit of the blood in the circulation, already shows that all or nearly all of the epinephrin is lost. With larger quantities, similarly injected, a loss was found if blood is obtained within $\frac{1}{2}$ to 1 minute, the blood showing approximately $\frac{1}{2}$ to $\frac{1}{4}$ of the theoretical concentration. If systemic blood is collected 2 or more minutes after just such a single dose there is rarely any epinephrin detectable in the blood by a sensitive test object. This indicates that epinephrin is lost rapidly in the circulation, probably by oxidation or some other form of destruction or by being fixed in the structures upon which it acts.

The present series of experiments was performed by injections of various doses of epinephrin constantly at a uniform rate for periods ranging from 13 minutes to 1 hour and 34 minutes.

Thirty-two (32) experiments were performed on 15 dogs, divided into 4 different groups. Briefly:

- Group 1. Specimens collected from the heart at intervals during and after cessation of the injection.
- Group 2. Specimens collected simultaneously during the injection from the aorta and from the vena cava.
- Group 3. Specimens collected simultaneously from the right heart and a femoral artery during the injection.
- Group 4. Specimens collected, within 1-3 minutes of each other, from the right and left hearts.

The amounts of epinephrin injected corresponded to 10-50 times the average normal rate of liberation from the adrenals under ordinary experimental conditions as determined by Stewart and Rogoff (12). A sample condensed protocol of an experiment in each group will suffice to illustrate the general results obtained (3 given in group 1).

Group 1:

Studies on non-anesthetized and anesthetized dogs on the presence of epinephrin in systemic blood during and after the cessation of the constant injection of epinephrin. Specimens taken from the heart.

(a) 17 experiments, with constant injections of 10-50 times average normal rate of liberation in three different unanesthetized dogs, for from 13 minutes to 1 hour and 34 minutes.

11 on #40
3 on #44
3 on #52

(b) 2 constant injections of the same type on two anesthetized dogs (#43, #90).

Sample condensed protocols of group 1:

Dog #40 on 7-30-36, M, weight 13.2 kg. No anesthesia. Injection for 22 min. of 0.13 mgms. epinephrin HCl per minute, corresponding to 50 times normal output. Specimen I from the heart was taken 20 min. after start of the injection with the injection in progress. Specimen II was taken 18 min. after cessation of the injection. Specimen I was assayed at a concentration somewhere between 1:100,000,000 and 1:200,000,000. Specimen II gave a small reaction; it was definitely less than 1:400,000,000.

Dog #40 on 9-11-36. No anesthesia. Injection of 0.024 mgms/min. of epinephrin HCl for 1 hr. and 34 min. This corre-

sponds to 10 times the normal rate of liberation from the adrenals. Specimen I was taken from the heart 70 min. after start of the injection with the injection in progress. Specimen II was removed 12 min. after cessation of the injection; there was some struggling and restlessness at the time II was taken. This assay was performed on an excellent intestinal segment. Both I and II, indistinguishable from each other, definitely contained less, if any at all, than a concentration of 1:1 billion, 600 million epinephrin HCl.

Dog #43, F, weight 10.1 kg. Nembutal anesthesia. Injection for 22 min. of 0.1 mgm/min. of epinephrin HCl; this corresponds to 50 times the normal rate of liberation. Specimen I, from the femoral artery, was taken 15 min. after start of the injection, with the injection in progress. Specimen II from the same place was taken 22 min. after cessation of the injection. Specimen I was assayed at a concentration somewhere between 1:50- and 1:60,000,000; specimen II was assayed at a concentration definitely less than 1:800,000,000.

Discussion of the Results Obtained in Group I

As mentioned above, the epinephrin concentrations in the blood were determined by the method of Stewart and Rogoff. Of all the available methods for detecting epinephrin in blood, this is the most sensitive, using the rabbits' intestine segment as a test object. With this method, if properly employed, one can detect 1:100,000,000 with almost every segment; it is not uncommon to have segments detecting 1:200,000,000 and even down to 1:800,000,000; occasionally one finds a segment that can detect a concentration of less than 1:1 billion.

In these experiments will be found good evidence on the rate of disappearance of epinephrin from the circulating blood. With amounts equivalent to 10 times the normal rate of epinephrin liberation from the adrenals, injected constantly, the epinephrin cannot be detected in the blood immediately after cessation of the injection; during the injection a trace may sometimes be found, and this only if the test object is extremely sensitive. The following table shows the concentrations found in the various experiments during and after the injections. Double figures indicate ranges of concentrations.

Dosage	Concentrations During Injection	Concentrations From Cessation of Down to 20 Minutes After Injection	Concentrations 25-30 Minutes After Cessation of Injection
With 10 x N	1:800,000,000 or less	None	None
With 15 x N	1:400,000,000 1:200,000,000	1:800,000,000 or less	None
With 25-30 x N	1:200,000,000 1:100,000,000	1:800,000,000 1:400,000,000	None
With 50 x N	1:100,000,000 1: 50,000,000	1:800,000,000 1:200,000,000	None

Thus, e.g., in one of the experiments (#52); weight 12.8 kg. Injection of 0.038 mgms/min., corresponding to 15 times the normal rate of liberation from the adrenals, the specimen collected during the injection, collected 22 minutes after beginning of the injection (I) was assayed at 1:200,000,000. The specimen taken 10 minutes after the end of the injection (II) was assayed at 1:800,000,000. After 22 minutes there had been introduced into the animal 22 x 0.038-0.836 mgms. of epinephrin. Theoretically, in a dog of this weight there would be about 1 kg. of blood and, if no epinephrin had been lost, there should have been an epinephrin concentration of about 1:836,000. There was about 1/200 of that amount, corresponding to 0.0041 mgms., and 10 minutes after the end of the injection at most only 1/800, corresponding to 0.001 mgms.

These results confirm the conclusions drawn above with regard to the experiments performed on the liberation of epinephrin from the adrenals--namely, that epinephrin is rapidly lost in the circulation. Obviously, this holds true even for constant injections, though with larger doses there is a larger residual concentration in the circulation. This may indicate that the destruction or fixation of epinephrin in the tissues proceeds only up to a more or less definite maximum quantity; all in excess of this quantity accumulates in the circulation and constitutes the residual concentration of epinephrin found at as-

say. The transitory "apparent" increase output of epinephrin (in the previous experiments) is thus satisfactorily explained by the presence of the residual injected epinephrin in the systemic circulation.

Though it is well known that pharmacological effects of epinephrin in the blood stream are quite transitory, evidence has been lacking to show, by quantitative experiments, that this is due to rapid disappearance of epinephrin when introduced into the circulation.

De Vos and Kochmann in 1905 (13) injected rabbits with single doses of epinephrin, withdrew blood from a carotid artery and injected it into the jugular vein of another rabbit, relying on its pressor effect. They found that 0.7 mgm/kg. injected into a rabbit could be detected only in the first 10 minutes after the injection. It should be noted that this dose corresponds to 3500 times the normal output from the adrenals for one minute--but, since a single injection requires only approximately 10 seconds, this dose corresponds to about 20,000 times the normal rate of liberation for a period of 10 seconds. With two-thirds of this dose they could no longer detect the injected epinephrin in 5 minutes; with one-third of the above dose they could no longer detect the injected epinephrin in 3 minutes.

Jackson in 1909 (14) was unable to demonstrate the presence of epinephrin, after injection into the circulation of dogs, as soon as the blood pressure returned to normal. His doses were of the magnitude of 1 to 2 cc. of 1:1,000 epinephrin. These amounts correspond to 500 to 1000 times the normal output of a 10 kg. dog if injected uniformly over a period of one minute; since they were single injections they really correspond to about 3000 to 6000 times the normal rate of liberation from the adrenals over a period of about 10 seconds. He attempted to detect the presence of epinephrin in the arterial blood by (a) reinjection into the same animal; (b) transfusion into another dog. He was successful in demonstrating epinephrin in the arterial blood only during the blood pressure rise of the injected animal, i.e., the donor. He found that if the transfusions were made during the height of the donor's blood pressure rise, the recipient's blood pressure exhibited an elevation; if the transfusions were made during the descent of the donor's blood pressure curve, the recipient's blood pressure showed (1) a rise in blood pressure,

(2) a fall in blood pressure, or (3) a rise, then a fall in blood pressure.

Trendelenburg, 1910 (15), was unable to demonstrate any epinephrin in circulating blood after the return of the blood pressure to normal. He employed the frog perfusion method.

Weiss and Harris, 1904 (16), by the same type of transfusion experiments employed by Jackson, claimed to have been able to demonstrate the presence of injected epinephrin, in cats, some minutes after the vasoconstriction had passed in the donor cat. They further claimed to have confirmed their results on a very unreliable method in frogs. Ehrman, 1905 (17), obtained results similar to those of Weiss and Harris, using the mydriasis of the frog's eye as an indicator. This has been shown by Schultz (18) to be a very unreliable method.

It becomes necessary at this point to indicate the difference between all the indirect evidence previously accumulated, on the one hand, and the direct evidence herein reported, on the other. A perusal of the literature clearly discloses the fact that there has never been any agreement whatsoever as to what constitutes a small dose or a large dose. Different workers compare results with those of others regardless of the amounts of epinephrin employed, or without taking into account the kind and size of animal used. With but few exceptions, practically all of the work on the disappearance of epinephrin from the circulation has been done with strictly pharmacological quantities of the drug. It cannot too strongly be emphasized that it is unsound to attempt physiological interpretations from pharmacological experiments. It has already been indicated that the amounts and concentrations in these experiments are always with reference to the basic physiological average normal output of 0.0002 mgm/kg/min. as determined by Stewart and Rogoff under ordinary experimental conditions. Thus, for a small dose we have chosen, when constantly injected, from 10 to 50 times the average normal rate of liberation from the adrenals.¹ This range is really not very

¹It has been found that constant injections of amounts that fall within these limits cannot be continued indefinitely with impunity. In two dogs such constant injections (with about 25 times the normal rate of liberation) were performed over a period of from 6 to 9½ hrs. At the end of those periods both animals were autopsied, and it was found that there were petechial hemorrhages throughout all the viscera, especially severe (in both

small, since it includes any amount of epinephrin that the adrenals can be forced to secrete by the maximal known stimuli (splanchnic nerve stimulation; strychnine and stimulation phase of nicotine). For a very large dose, 100 to 500 times the average normal rate of liberation, has been employed.

None of the previously used methods are sensitive enough to detect some of the epinephrine concentrations encountered in this work. As a group, the chemical methods fall far short of the necessary sensitivity. The frog perfusion method, employed by Trendelenburg, O'Connor, and others, can yield some information, as a test object, chiefly of a qualitative nature. It is not sensitive enough for quantitative work, since pressor substances are rapidly formed in shed blood, as Trendelenburg has shown in 1910. Elliott's pithed cat preparation is perhaps the best of the blood pressure methods; essentially it is a preparation in which blood pressure effects are obtained from a spinal pressure level, a condition which allows of a more faithful response than that of trying to obtain blood pressure reactions superimposed on a normal blood pressure. However, it also falls short of the sensitivity required in this work.

In the foregoing literature one finds only indirect indications that epinephrin is rapidly lost, e.g., by the evanescence of pharmacological effects. Herein is presented evidence of a direct nature by virtue of the fact that actual measurements of epinephrin concentrations were made.

What happens to the epinephrin that is quite evidently lost from the circulation is, in itself, a problem. It cannot be the alkalinity of the blood that destroys it, for Stewart and Rogoff showed that epinephrin in blood is fairly stable (in vitro) for considerable lengths of time. Elliott and Durham (19) were unable to demonstrate the formation of antibodies to epinephrin. The possibility of excretion, via the kidneys, is not great, for even those workers that found traces in the urine after large doses of epinephrin used chemical methods which are not reliable. Also the duration of the visible effects of the injected epinephrin is not altered by renal ligation (Jackson).

cases) in the myocardium. A similar observation has recently also been reported by Dragstedt, Prohaska, and Harms (meetings of the American Physiological Society, 1937).

The elimination of the above possible reasons for loss of epinephrin from the circulating blood leaves one more possibility--namely, that of oxidation or fixation of the epinephrin by the tissues. Very little evidence exists that the tissues really utilize the injected epinephrin in some manner. Tatum, 1912 (20), showed that there is some loss of epinephrin from an epinephrin solution in which arterial rings were suspended, especially if a constant stream of oxygen is passed through the solution. Accordingly, the following experiments were performed in an attempt to uncover further evidence that the tissues do utilize epinephrin in the circulating blood.

Group 2:

Four experiments on 3 anesthetized animals (91; 102; 2 on 103) were performed, with constant injections of 15 to 30 times the average normal rate of liberation of epinephrin from the adrenals (into the external jugular vein). Specimens were collected simultaneously from the aorta and vena cava (at the level of the hilus of the kidney) in $1\frac{1}{2}$ to 12 minutes after beginning of the injection. These experiments were designed to see if one could detect whether, and how much, epinephrin may be used by the tissues. The above mentioned sites of collection were chosen because if one can show a difference in concentration in these two vessels after the first circuit of blood, the only logical conclusion is that some is lost somewhere between the two points of collection. It was found that the concentration of epinephrin in the vena cava with these injections was about one-half to two-thirds that found in the aorta when bloods are collected simultaneously.

Sample condensed protocol of an experiment in this group:

Dog #103, male, weight 11.0 kg. under urethane anesthesia, received a constant injection of 0.066 mgm/min., corresponding to 30 times his normal rate of output. Specimen aorta, III, and specimen vena cava, IV, were collected simultaneously $2\frac{1}{2}$ min. after the beginning of the injection. Specimen III assayed at between 1:80,000,000 and 1:100,000,000; specimen IV assayed at 1:150,000,000 to 1:200,000,000.

If no loss had taken place during the $2\frac{1}{2}$ minutes, the concentration of epinephrin in the blood at the end of that period should have been 1:5,000,000 to 1:6,000,000, i.e., $0.066 \times 2\frac{1}{2}$, or 0.165 mgms. in about 900 cc. of blood; whereas the experimental concentration of 1:100,000,000 amounts to only 0.009 mgm. present

in the circulating blood. We see, therefore, that not only has there been some disappearance, but that each circuit sees the loss of yet more.

Group 3:

Five experiments (78, 79, 80, 81, 82) with constant injections in anesthetized animals of 12 to 58 times normal rate of output for the animal. Simultaneous collection of specimens from the right heart and a femoral artery were taken in from 1 to 2 minutes after beginning of the injection and again in from 10 to 12 minutes after beginning of the injection.

Though these assays were not particularly well quantitated, qualitatively their story is genuine--namely, that the right heart specimens exhibited a greater concentration of epinephrin than did the femoral artery specimens.

Sample condensed protocol of an experiment of group 3:

Dog #79, weight 8.0 kg., who received a constant injection of 58 times his normal output per minute (femoral v.)--one minute after the injection was begun specimen I (femoral artery) and specimen II (right heart) were taken simultaneously; 9 min. later specimen III (fem. a.) and specimen IV (right heart) were again collected simultaneously. The assay was not quantitative in this case, but II was definitely and consistently greater than I; likewise IV was definitely and consistently greater than III.

From these experiments it seems but reasonable to conclude that blood, in going through the pulmonary circuit, loses a portion of its contained epinephrin. It has previously been thought that no epinephrin was consumed in its passage through the lungs, based originally on the presumed absence of vasomotor nerves from this structure.

Group 3:

Four experiments on 2 un-anesthetized animals (2 on 40; 2 on 52). Constant injections were administered, and in from 14 to 28 minutes specimens were taken, one from the right heart, one from the left, within one to 3 minutes of each other; in some cases the right-, in some the left-heart, specimen was taken first.

Sample condensed protocol of an experiment in this group:

In one of the experiments on dog 52, in which the dog received a constant injection of 50 times her own rate of output, a specimen from the right heart (I) was taken 24 min. after start of the injection; II, from the left heart, was collected 3 min.

later, and another specimen III taken 4 min. after the end of the injection. This assay was well quantitated, viz.:

Concentration of epinephrin in I - 1:50,000,000

Concentration of epinephrin in II - 1:100,000,000

Concentration of epinephrin in III - less than 1:400,000,000

After 24 minutes of the injection there should have been an epinephrin concentration in the blood of about 1:350,000-or-3.0 mgms. ($0.0025 \times 50 \times 24$ min.). This, assuming about 1 kg. of blood. At 1:500,000,000 there was 0.02 mgms. in the blood, or 1/150 of the amount that would have been there, had there been no loss.

This experiment was confirmed qualitatively by one of the other experiments on 40. In one other such experiment (on 52) the two heart specimens could not be distinguished; in still another (on 40) the arterial specimen proved to be of a slightly greater epinephrin concentration than the venous specimen. The latter assay was not particularly reliable. It is to be remembered, of course, that the withdrawal of a specimen should be made with a constant rate, and it is possible that one might withdraw blood as yet not well mixed from the heart, thus giving a spurious result.

These experiments lend further support to the fact that epinephrin is removed in the pulmonary circuit, and to the removal of epinephrin from the systemic blood.

CONCLUSIONS

1. Intravenous injection of epinephrin does not cause an increased rate of liberation of epinephrin from the adrenals.

2. Increased concentrations of epinephrin in adrenal vein blood, following injections of large quantities of epinephrin, correspond with increased concentrations of epinephrin in the systemic blood, and does not indicate an increase in the rate of liberation from the adrenals.

3. A large dose of epinephrin, injected intravenously, may lead to a moderate transient diminution in the epinephrin output from the adrenals after the pressor effects of the injected epinephrin have subsided.

4. Injected epinephrin is rapidly lost from the circulating blood. Within 1/2 to 2 minutes after the end of a single in-

jection, according to dose, it can no longer be detected in the blood with sensitive test objects. After prolonged constant injections, with large doses, some epinephrin may still be detected for periods up to about 20 minutes.

5. Evidence has been obtained by quantitative measurements, showing that epinephrin is speedily lost both in the pulmonary and systemic circulations.

6. Intervention at the adrenals as a therapeutic measure in hypertension does not have the support of unequivocal quantitative experimental studies.

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